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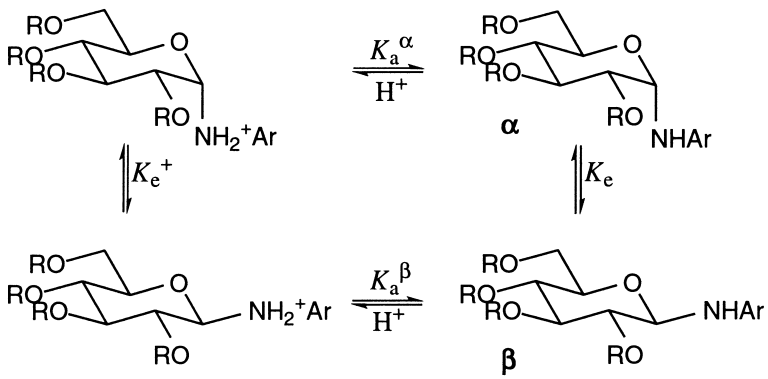

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Anomeric Effects versus Steric Hindrance to Ionic Solvation in Protonated Glucosylanilines and Cyclohexylanilines

Charles L. Perrin* and Joshua Kuperman

Contribution from the Department of Chemistry, University of California−San Diego, La Jolla, California 92093-0506

Received April 24, 2003; E-mail: cperrin@ucsd.edu

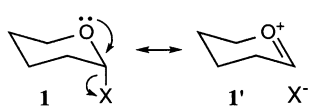
Abstract: The so-called reverse anomeric effect is the preference of cationic substituents for the equatorial position on a pyranose ring, but it is not consistent with current theories of molecular structure or with previous studies designed to test it. To probe this further, the N-protonation-induced shifts of the anomeric equilibrium in a series of *N*-(tetra-*O*-methylglucopyranosyl)anilines have been measured with high precision through an NMR titration method that compares basicities of α and β anomers in a mixture of the two. For comparison, the N-protonation-induced shifts of the cis/trans equilibrium in *N*-(4-*tert*-butylcyclohexyl)anilines have also been measured by this same method. In both series, there is a shift of the equilibrium toward equatorial upon N-protonation, consistent with steric hindrance to ionic solvation. This shift is smaller for the glucosylanilines than for the cyclohexylanilines, consistent with an enhancement of the normal anomeric effect that counters the steric hindrance and reduces the shift toward the equatorial β anomer. Moreover, the shift toward equatorial increases slightly but detectably with electron-withdrawing substituents on the cyclohexylaniline, which fine-tune the steric hindrance to ionic solvation. In contrast, the shift decreases for the glucosylanilines. This is consistent with an enhancement of the normal anomeric effect due to a more localized positive charge, rather than with a reverse anomeric effect. These results thus define the substituent dependence of the anomeric effect.

Introduction

Reverse Anomeric Effect. The steric preference of a substituent X for the equatorial position on a six-membered ring is well-known. A quantitative measure of this preference is designated as A_X, the free-energy difference between axial and equatorial conformers of a cyclohexane (eq 1).[1] For substituents bulker than hydrogen, this value is greater than zero. The

$$A_X = G_{\text{axialX}}^\circ - G_{\text{equatorialX}}^\circ = -RT \ln([\text{axialX}]/[\text{equatorialX}]) \quad (1)$$

anomeric effect is the countertendency for an electronegative X at C1 of a tetrahydropyran derivative (**1**) to take the axial position.[2] It is believed to be due to an $n_O-\sigma_{C-X}^*$ delocalization that stabilizes the axial form[3] or, equivalently, to the contribution of a charge-separated double-bond/no-bond resonance form (**1′**).



When X is cationic, the equilibrium has been claimed to shift toward equatorial, a phenomenon called the reverse anomeric effect (RAE).[4] The term has had other connotations, but we here restrict it to cationic substituents. This has raised considerable controversy and skepticism, because the positive charge ought to lower the energy of the σ_{C-X}^* orbital and enhance the stabilization of the axial form, not reverse it. Alternatively, resonance form **1′** ought to contribute even more when the substituent is cationic, because there is no longer the penalty for charge separation.

According to one critical review of the RAE,[5] the evidence was in conflict. The first examples were α-glycosylpyridinium ions, where the results could have been due merely to steric repulsions. The clearest evidence for the RAE was the claim that the equatorial preference in a xylosylimidazolium ion exceeds what can be attributed to steric factors.[6] The data, obtained from NMR coupling constants, are reproducible and not due to changes in solvent polarity, but the RAE was nevertheless rejected in favor of steric effects.[7] The RAE is definitely not operative in protonated glucosylamines, with NH_2R^+ groups of known steric contribution.[8] It was therefore

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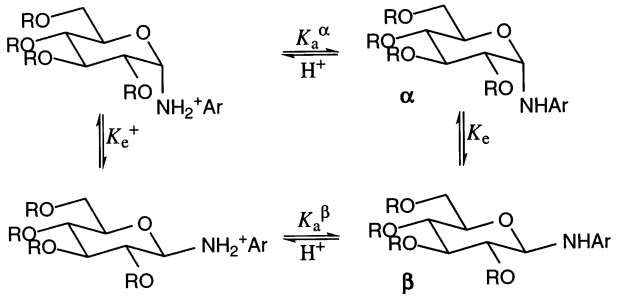
concluded that coupling constants could not measure xylosylimidazole populations reliably. Additional experimental evidence is sparse,[9] although geometric changes are consistent with an enhanced normal anomeric effect, not a reverse one.[10] Molecular orbital calculations are not conclusive,[11] because it is difficult to separate the RAE from steric effects and hydrogen bonding, which also favor an equatorial form.

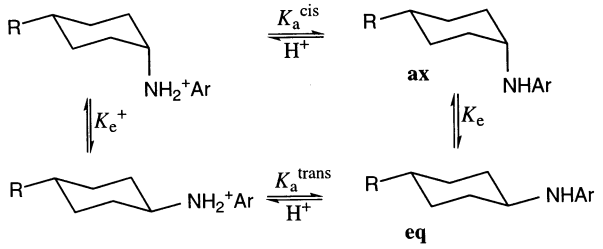
It is important to understand the conformational behavior of sugar derivatives with cationic groups at the anomeric center. Many biomolecules have cationic or protonatable heterocyclic bases attached to a sugar, the most familiar being NAD^+ and the conjugate acids of nucleosides. Many other sugar derivatives and analogues react only when protonated, and the conformation of such activated intermediates is crucial for assessing stereoelectronic effects.[12] Moreover, understanding the conformational behavior of such cations can guide stereospecific syntheses of sugar derivatives.[13] It must be noted though that our concern is only with the endo anomeric effect, not the exo, which affects the conformation about the exocyclic C1−X bond of the unprotonated species and is operative in both α and β anomers.[14]

We therefore had reinvestigated glycopyranosylimidazoles. The RAE would be manifested as an increase in the proportion of the β anomer on N-protonation of an equilibrating mixture. Although glycosylimidazoles are configurationally stable and do not equilibrate,[15] the increase can be evaluated indirectly, because a corollary of the RAE is that the β anomer must be more basic than the α. Instead, we found the opposite.[16] Similarly, the greater basicity of α-glucosylamine, measured by direct titration,[17] can now be recognized as being inconsistent with the RAE.

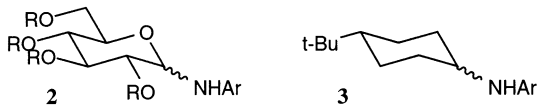
Scheme 1. Acid Dissociations of α and β Tetramethylglucopyranosylanilinium Ions (R = CH_3)



Scheme 2. Acid Dissociations of Cis and Trans 4-*tert*-Butylcyclohexylanilinium Ions (R = $(CH_3)_3C$)



Glucosylanilines. This paper compares the anomeric equilibria of protonated and unprotonated tetra-*O*-methylglucosylanilines (**2**, R = CH_3). Again, the RAE would be manifested as an increase in the proportion of the β anomer on N-protonation of an equilibrating mixture. In terms of Scheme 1, this means that K_e^+ would be greater than K_e. It must be recognized that Scheme 1 constitutes a thermodynamic cycle. It then follows that K_e^+/K_e must equal the ratio of acidity constants, K_a^{α}/K_a^{β}. Thus, the RAE would equivalently be manifested as a greater acidity of the α anomer or a greater basicity of the β.



Steric hindrance to ionic solvation must also be taken into account in comparing protonated and unprotonated glucosylanilines. This phenomenon is well established, as in the basicities of the methylated amines and the acidities of alcohols.[18] For imidazolyl, the steric contribution is small,[19] because protonation occurs at a distant nitrogen. Of course this cannot be general, and steric hindrance to ionic solvation could strongly disfavor protonation of the α anomer of **2**.

The extent to which the positive charge of NH_2Ar^+ disfavors an axial stereoisomer can be assessed with 4-*tert*-butylcyclohexylanilines (**3**). A measure of steric hindrance to ionic solvation is ΔA, the difference between the A values (eq 1) of NH_2Ar^+ and NHAr substituents. This is equal to $-RT \ln(K_e^+/K_e)$, where K_e^+ and K_e are equilibrium constants in Scheme 2. Because this scheme likewise constitutes a thermodynamic cycle, ΔA must also equal the ratio of acidity constants, $K_a^{cis}/$

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K_a^{trans}. Thus, steric hindrance to ionic solvation would equivalently be manifested as a greater acidity of the axial stereoisomer or a greater basicity of the equatorial. For quantitative comparison, it must be recognized that this ΔA is an underestimate of the steric contribution in glucosylanilines, because steric effects in tetrahydropyrans are more severe than those in cyclohexanes.[20] Moreover, it is uncertain what effect the 2-methoxy in **2** might have.

What makes the current study feasible is an NMR titration method that measures relative basicities of closely related molecules without the necessity of separating them.[21] The ratio of acidity constants in Scheme 1 or 2 (K_a^{α}/K_a^{β} or K_a^{cis}/K_a^{trans}) is obtained as the slope K_a^{ax}/K_a^{eq} of a linearized plot (eq 2), created from the observed chemical shifts (δ_{ax},δ_{eq}) of reporter nuclei, measured after successive additions of aliquots of acid to a mixture of the two bases. Also needed are the chemical shifts of the neutral (δ_{ax^0},δ_{eq^0}) and protonated forms (δ_{ax^+},δ_{eq^+}), which can be measured at the beginning and end of the titration and which must differ appreciably if the nuclei are to be appropriate as reporters. Because no pH measurement is necessary, the method is capable of exceptionally high precision.

$$(\delta_{eq} - \delta_{eq^0})(\delta_{ax^+} - \delta_{ax}) = (K_a^{ax}/K_a^{eq})(\delta_{ax} - \delta_{ax^0})(\delta_{eq^+} - \delta_{eq}) \quad (2)$$

Because Scheme 1 or 2 each constitutes a thermodynamic cycle, the relative basicities then provide an indirect determination of the ratio K_e^+/K_e. This ratio represents the extent to which N-protonation shifts the equilibrium from axial toward equatorial. For glucosylanilines (**2**), the ratio can be converted to $\Delta\Delta G_{\beta\rightarrow\alpha}^{\circ}$, the change upon N-protonation of the free-energy difference ΔG° between α and β anomers (eq 3). Similarly, the ratio for a *tert*-butylcyclohexylaniline (**3**) can be converted to ΔA, the difference between *A* values (eq 1) of NH_2Ar^+ and NHAr substituents (eq 4).

$$RT\ln(K_e^+/K_e) = \Delta\Delta G_{\beta\rightarrow\alpha}^{\circ} = \Delta G_{NH_2Ar^+}^{\circ} - \Delta G_{NHAr}^{\circ} = RT\ln(K_a^{\alpha}/K_a^{\beta}) \quad (3)$$

$$RT\ln(K_e^+/K_e) = \Delta A = A_{NH_2Ar^+} - A_{NHAr} = RT\ln(K_a^{cis}/K_a^{trans}) \quad (4)$$

Moreover, it is possible to vary the substituents on the aromatic ring. Electron-withdrawing substituents localize and intensify the positive charge on the protonated nitrogen. This permits a sensitive test of the consequences of N-protonation. To the extent that a positive charge leads either to a RAE or to an enhancement of the normal anomeric effect, and to the extent that a positive charge creates steric hindrance to solvation, these effects are expected to be larger with electron-withdrawing substituents. A convenient measure of electron-withdrawing power is the pK_a of the corresponding $ArNH_3^+$. Thus, it is possible not only to measure the effects of N-protonation on the anomeric equilibrium in glucosylanilines (**2**) and on the cis/trans equilibrium in *tert*-butylcyclohexylanilines (**3**), but also to fine-tune the amount of proximal positive charge that is generated by protonation. Indeed, our goal was not primarily to disprove the RAE, because earlier results cast considerable doubt upon it, but rather to define quantitatively how substituents modify the anomeric effect.

This study complements another on a similar series of tetraacetylglucopyranosylanilines (**2**, R = CH_3CO).[22] According to NMR integrations, the anomeric equilibrium shows small variations with aromatic substituent and solvent. Acidic conditions shift the equilibrium toward the β anomer, but no monotonic relationship could be detected between that shift and the electron-withdrawing power of the substituent, and the shift in the corresponding 5-thio analogues is toward the α anomer. It was concluded that N-protonation of the parent glucosylaniline leads to anomeric stabilization of the α anomer by ~1 kcal/mol, which is opposed by a steric destabilization of ~1.2 kcal/mol. The RAE was rejected, and the irregular variations were ascribed to a combination of steric effects, electrostatic effects, and orbital interactions. We now report NMR titration results that provide a consistent assessment of both anomeric and steric effects in a series of glucosylanilines, as well as the substituent effects.

Experimental Section

Tetramethylglucopyranosylanilines (2). Glucosylanilines themselves were found to be unsuitable for these experiments, because they hydrolyze in aqueous acid and they are insufficiently soluble in aprotic solvents. Although the tetra-*O*-acetyl derivatives remain stable in nonaqueous media, the acetyl signals mask the NMR signals of the anomeric hydrogens. Tetra-*O*-methylglucosylanilines proved to be suitable. The anomeric signals are sufficiently isolated, allowing accurate assignment and measurement of the chemical shifts. It must be admitted that the experiments were still complicated by decomposition and NMR line broadening, especially as the basicity of the aniline decreased. Consequently, this study could not be extended to more electron-withdrawing substituents, such as nitro.

In a typical procedure,[23] tetra-*O*-methylglucose[24] (1 mmol) and the desired aniline (2 mmol) were dissolved in minimum ethanol and refluxed for at least 4 h. The solution was cooled over ice, and the resulting crystals were filtered off and rinsed with cold ethanol. Only the *para*-methoxy product needed to be recrystallized. To minimize hydrolytic decomposition during the subsequent titrations, water was removed by lyophilization: mp 152−153° (*p*-BrC_6H_4), lit.[25] 154°, mp 97−98° (*m*-$CH_3OC_6H_4$), mp 120−122° (*p*-FC_6H_4), mp 134−135° (C_6H_5), lit.[25] 135°, mp 149−150° (*p*-$CH_3C_6H_4$), lit.[25] 151°, mp 109−110° (*p*-$CH_3OC_6H_4$), lit.[25] 110°.

4-*tert*-Butylcyclohexylanilines (3). A procedure for cyclohexylamines was adapted.[26] 4-*tert*-Butylcyclohexanone (3.08 g, 0.02 mol) was combined with the appropriate aniline (0.04 mol) and catalytic *para*-toluenesulfonic acid in benzene. The mixture was refluxed using a Dean−Stark trap until no more water condensed. The benzene was removed by distillation, and methanol, $NaBH_3CN$ (3.77 g, 0.06 mol), and a small amount of bromocresol green were added. Concentrated HCl was added dropwise until the solution remained pale yellow. The mixture was then stirred for 20 h, diluted with water, made basic with KOH, and extracted with ether. The organic layer was dried with K_2CO_3, and the solvent was removed at reduced pressure, yielding the desired mixture of cis and trans 4-*tert*-butylcyclohexylanilines. The ^1H

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Table 1. K_a^{α}/K_a^{β} for Substituted Tetra-*O*-methylglucosylanilines (**2**)

subst	$\delta_{\alpha}{}^{0}$, ppm	$\delta_{\beta}{}^{0}$, ppm	K_a^{α}/K_a^{β}	$\Delta\Delta G_{\beta\rightarrow\alpha}{}^{\circ}$, cal/mol
p-Br	5.16	4.46	1.61 ± 0.05	281 ± 18
m-CH_3O	5.21	4.52	1.68 ± 0.06	308 ± 22
p-F	5.15	4.46	1.69 ± 0.03	312 ± 12
H	5.21	4.53	1.86 ± 0.04	366 ± 14
p-CH_3	5.16	4.49	2.09 ± 0.04	437 ± 11
p-CH_3O	5.12	4.44	2.08 ± 0.03	434 ± 8

Table 2. K_a^{cis}/K_a^{trans} for Substituted 4-*tert*-Butylcyclohexylanilines (**3**)

subst	$\delta_{cis}{}^{0}$, ppm	$\delta_{trans}{}^{0}$, ppm	K_a^{cis}/K_a^{trans}	ΔA, cal/mol
p-Cl	6.60[a]	6.56[a]	3.52 ± 0.03	745 ± 6
H	3.62	3.16	3.21 ± 0.04	692 ± 8
p-CH_3	3.58	3.10	3.17 ± 0.03	683 ± 6
p-CH_3O	3.54	3.06	3.07 ± 0.06	665 ± 11

[a] For ortho H.

NMR spectrum of the parent (Ar = phenyl) agrees with that of the recrystallized trans isomer,[27] except that it shows the presence of cis. If product appeared to be sufficiently pure, recrystallization was avoided, so as to retain the cis: mp 128° (*p*-ClC_6H_4), mp 108−109° (C_6H_5), lit.[28] 108−109°, mp 124−126° (*p*-$CH_3C_6H_4$), mp 69−72° (*p*-$CH_3OC_6H_4$), lit.[28] 77−78°.

NMR Titrations. Acetonitrile was chosen as the solvent for these titrations because the methylated sugars are soluble and stable, and the H1 signals undergo measurable chemical-shift changes upon protonation. Triflic acid-*d* was chosen because it is anhydrous and was found to be strong enough to protonate the glucosylanilines.

Stock solutions of acid contained 0.23 mmol of CF_3SO_3D in 1.0 mL of acetonitrile-d_3. In a typical titration, 0.023 mmol of a mixture of tetramethylglucosylaniline or cyclohexylaniline stereoisomers was dissolved in 0.7 mL of acetonitrile-d_3 plus tetramethylsilane as internal standard. For glucosylanilines, a trace of stock acid was added to catalyze anomerization. Acid was then added to each NMR sample in 10-μL aliquots, and at least 10 additions were made. The chemical shifts of an appropriate reporter nucleus in each of the two stereoisomers were measured after each addition. Titrations were assumed to be complete when further acid resulted in no change in chemical shifts.

The observed lack of further change is evidence that triflic acid is indeed strong enough to protonate the weakly basic tetramethylglucosylanilines, even in a solvent of intermediate polarity. This avoids the reliance on aqueous pK_a's of triflic acid and of the parent $ArNH_3^+$ for judging that 1.5 equiv of triflic acid would ensure complete proton transfer to such weakly basic glucosylanilines, even in so nonpolar a solvent as CD_2Cl_2.[22]

Results

Titrations of Glucosylanilines. The K_a^{α}/K_a^{β} values obtained from titrations of various tetra-*O*-methylglucosylanilines (**2**) are reported in Table 1. Included are the initial chemical shifts of the H1 reporter nuclei that were followed for each of the two anomers, as well as the conversions to $\Delta\Delta G_{\beta\rightarrow\alpha}{}^{\circ}$ (eq 3).

Titrations of Cyclohexylanilines. Table 2 reports the K_a^{cis}/K_a^{trans} and ΔA (eqs 2,4) values obtained from titrations of 4-*tert*-butylcyclohexylanilines (**3**). Included are the initial chemical shifts of the reporter nuclei that were followed (H1 unless otherwise noted). The chemical shifts of the axial H1 in the trans stereoisomer and of the equatorial H1 in the cis are designated δ_{trans} and δ_{cis}, respectively.

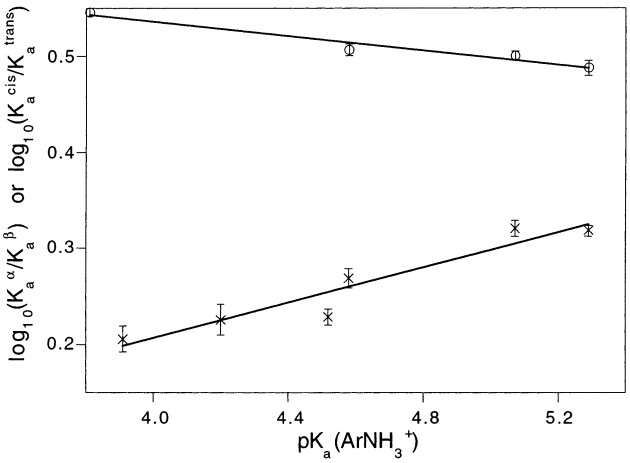

Figure 1. Plot of $\log(K_a^{\alpha}/K_a^{\beta})$ of tetramethylglucosylanilines (×) and $\log(K_a^{cis}/K_a^{trans})$ of 4-*tert*-butylcyclohexylanilines (○) versus pK_a of the corresponding $ArNH_3^+$.

Errors. Titrations of cyclohexylanilines gave K_a^{cis}/K_a^{trans} with reasonable errors, as was judged from statistical analysis of the fit to eq 2. Titrations of glucosylanilines were less reproducible, and K_a^{α}/K_a^{β} was obtained as a weighted mean from replicate titrations. In both cases, the errors reported in the tables are remarkably low.

Substituent Effects. Figure 1 shows $\log(K_a^{ax}/K_a^{eq})$ for both the tetramethylglucosylanilines (**2**) and the *tert*-butylcyclohexylanilines (**3**), plotted against the pK_a of the aniline itself.[29] For the former, the data can be fit to a straight line, with a slope of +0.091 and with a correlation coefficient of 0.954. For the latter, the slope is −0.037, and the correlation coefficient is 0.977. Usually, low slopes lead to low correlation coefficients, but these are respectably high.

Discussion

Configurational Stability. Both the *tert*-butylcyclohexylanilines and the glucosylanilines are configurationally stable on the NMR time scale. The former were synthesized as a mixture of cis and trans stereoisomers, and they do not interconvert. The latter were obtained as crystalline β anomers, and a trace of acid was used to generate a small amount of α. Even though the amount may change as the titration proceeds, concentrations are never measured by signal integration. Instead, relative basicities are measured by NMR titration. The thermodynamic cycles of Schemes 1 and 2 then permit conversion to the change of equilibrium on N-protonation. It is perhaps counterintuitive that this change of equilibrium can be measured even though the equilibrium is never established. The feasibility of this "indirect" measurement is testimony to the power of thermodynamics.

RAE versus Enhancement of the Normal Anomeric Effect. The K_a^{α}/K_a^{β} and $\Delta\Delta G_{\beta\rightarrow\alpha}{}^{\circ}$ values in Table 1 are all positive, meaning that the β anomer is more basic than the α. Equivalently, it follows from Scheme 1 that K_e^+ is greater than K_e, meaning that N-protonation shifts the anomeric equilibrium in glucosylanilines toward β. This result would appear to support the operation of the RAE. However, these values are comprised

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of three possible contributions. There is a positive one from the increased steric bulk of a protonated substituent relative to the unprotonated, because of steric hindrance to ionic solvation. There may be an additional positive contribution from the RAE. Conversely, there may be a contribution from an enhancement of the normal anomeric effect, which would shift the equilibrium toward α and contribute negatively to $\Delta\Delta G_{\beta\rightarrow\alpha}^{\circ}$. Only if the observed $\Delta\Delta G_{\beta\rightarrow\alpha}^{\circ}$ exceeds the steric contribution could it be concluded that the positive value is evidence for the RAE.

The operation of a steric effect is apparent from the K_a^{cis}/K_a^{trans} values in Table 2, which are uniformly positive. This means that K_e^+ is again greater than K_e or that N-protonation shifts the equilibrium in these cyclohexylanilines also toward trans. Alternatively, the positive ΔA values reflect a greater effective steric bulk for a protonated aniline, which requires solvation and is thus more stable when equatorial. It is not necessary to specify the exact origin of this steric effect, or to ascribe it to syn-axial repulsions.[30] By whatever mechanism it operates, this steric hindrance to ionic solvation must also be operative in the glucosylanilines.

The ΔA values in the cyclohexylanilines provide an estimate of the steric contribution to $\Delta\Delta G_{\beta\rightarrow\alpha}^{\circ}$ in the glucosylanilines. Subtracting ΔA from the observed $\Delta\Delta G_{\beta\rightarrow\alpha}^{\circ}$ gives the anomeric contribution, separated from the steric. The average from those with substituents in common is −270 cal/mol. This is negative, representing an N-protonation-induced shift of the equilibrium toward α. Moreover, because steric effects in tetrahydropyrans are more severe than those in cyclohexanes,[20] this ΔA from cyclohexylanilines underestimates the steric contribution, and the magnitude of the anomeric shift toward α is even larger than 270 cal/mol.

The key result is the qualitative one that $\Delta\Delta G_{\beta\rightarrow\alpha}^{\circ}$ in glucosylanilines is smaller than ΔA in cyclohexylanilines. The difference, corresponding to an estimate of the anomeric contribution alone, is small but statistically highly significant. Thus, N-protonation makes the equatorial isomer more favorable for both, but to a greater extent for the cyclohexylaniline than for the corresponding glucosylaniline. This is the exact opposite of what would be expected from the RAE, and it is entirely consistent with an enhancement of the normal anomeric effect. Because anomeric effects represent the tendency of an electronegative substituent at C1 of a tetrahydropyran (**1**) to take the axial position, this tendency ought to be enhanced on protonation of that substituent, which augments its electronegativity.

We therefore reaffirm the conclusion that there is no firm evidence for the RAE. The enhancement of the normal anomeric effect is consistent with the molecular orbital interpretation of anomeric effects.[3] It is inconsistent with an electrostatic interaction between the charge on the protonated nitrogen and the oxygen dipole, which in principle might stabilize the equatorial form.[5]

Substituent Effects. The K_a^{ax}/K_a^{eq} values in Tables 1 and 2 vary with the aniline substituent. This is clear from Figure 1, where the electronic nature of the substituent is measured by the basicity of the parent aniline. The slopes are quite small, but they are measured reliably. Moreover, glucosylanilines and cyclohexylanilines respond oppositely. This phenomenon is more subtle than in the usual situation, where an equilibrium or rate responds to substituents that stabilize or destabilize charge that develops in the reaction. Here, both K_a^{ax} and K_a^{eq} respond nearly equally to substituents, because both reactions convert a cation into a neutral, and the slope represents the difference between the two responses. The substituents only fine-tune the amount of proximal positive charge that is generated by protonation.

The opposite signs for the slopes in Figure 1 are a consequence of the opposing origins for K_a^{α}/K_a^{β} and K_a^{cis}/K_a^{trans}. In both cases, an electron-withdrawing substituent localizes and intensifies the positive charge on the nitrogen. For cyclohexylanilines, this creates a requirement for additional solvation, which is subject to steric hindrance, so K_e^+/K_e increases, but for glucosylanilines, the greater positive charge increases the enhancement of the normal anomeric effect and decreases K_e^+/K_e. Moreover, the magnitude of the slope is greater for glucosylanilines, showing that the anomeric effect is more sensitive to the effective electronegativity of the substituent than is steric hindrance to ionic solvation. This is evidence that internal solvation by the 2-methoxy to both anomers of **2** is not leveling the steric hindrance to solvation.

This study goes beyond an earlier one on the anomeric equilibrium in tetra-*O*-acetylglucosylanilines.[22] Our results reinforce their conclusion that N-protonation does not produce a RAE but instead leads to an enhancement of the normal anomeric effect. With the 5-thio analogues, this was clear, because K_e ([β]/[α], as in Scheme 1) decreases on N-protonation. For the glucosylanilines, K_e instead increases on N-protonation, and there was no simple relationship between the basicity of the aniline and the increase of K_e. Both K_e^+ (in the protonated forms) and K_e^+/K_e were smaller for the parent glucosylaniline than for either the *para*-methoxy or the *para*-nitro derivative. This nonmonotonic behavior was attributed to a balance of anomeric and steric effects, but it disagrees with our results, because the thermodynamic cycle guarantees that the increase of K_e on N-protonation must be equivalent to our K_a^{α}/K_a^{β}, which Figure 1 shows varies regularly with basicity. Besides, the low slope for cyclohexylanilines in Figure 1 demonstrates only a small dependence of the steric effect on substituent. The data in Table 2 show how difficult it is to measure these substituent effects by NMR integrations, because the entire variation is from a 3.1:1 ratio to a 3.5:1 ratio. It is also possible that proton transfer to glucosylanilines was incomplete in nonpolar solvents, especially to the less basic ones. The NMR titration method provides consistent results that vary regularly with substitution.

Above it was asserted that electron-withdrawing substituents localize and intensify the positive charge on the protonated nitrogen. Certainly, they increase the amount of proximal positive charge in both neutral aniline and protonated anilinium ion, but is the increase really greater in the latter? Because resonance can delocalize electron density from the nitrogen lone pair of the former, electron-withdrawing substituents might instead produce a greater increase of positive charge in the neutral. However, the negative slope for cyclohexylanilines in Figure 1 shows that electron-withdrawing substituents create a greater steric hindrance to solvation, thus showing that they do indeed increase the amount of proximal positive charge generated by N-protonation.

The data in Figure 1 show small deviations from the straight-

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line fits. Some of these are due to random scatter. Another source is the inadequacy of aniline basicity in tracking the intensification of positive charge on the nitrogen, because these two phenomena reflect different mixes of inductive and resonance contributions. Still another possibility to account for deviations is curvature. There is no requirement for linearity, which is a default relationship. Indeed, extrapolation would suggest that the lines cross at pK_a 6.6. This corresponds to a hypothetical aniline with a substituent so electron-donating that the enhancement of the anomeric effect vanishes and only steric hindrance to solvation of a delocalized positive charge remains. If so, the curves would not cross but converge instead.

Conclusions

Through a highly precise NMR titration method that is applicable to a mixture of nonequilibrating stereoisomers, we have succeeded in measuring the N-protonation-induced shifts of the anomeric equilibrium in tetra-*O*-methylglucosylanilines and of the cis/trans equilibrium in 4-*tert*-butylcyclohexylanilines. Both equilibria shift toward the equatorial stereoisomer upon N-protonation ($K_e^+ > K_e$ in Schemes 1 and 2), a behavior consistent with steric hindrance to ionic solvation. However, the shift is smaller for the glucosylanilines than for the cyclohexylanilines. Therefore, some effect is countering the steric hindrance and reducing the shift of the equilibrium toward the equatorial β anomer. This is consistent with an enhancement of the normal anomeric effect, rather than a RAE. We reaffirm the conclusion that there is no firm evidence for this latter effect.

Moreover, the N-protonation-induced shift toward equatorial increases slightly with electron-withdrawing substituents on the cyclohexylaniline. This behavior is consistent with steric hindrance to ionic solvation. In contrast, electron-withdrawing substituents lead to a reduced shift toward equatorial (β) for the glucosylanilines, again consistent with an enhancement of the normal anomeric effect, and not with a reverse one. Both the steric hindrance to ionic solvation and the enhancement of the anomeric effect are greater when electron-withdrawing substituents localize and intensify the positive charge on the protonated nitrogen. These results thus define the dependence on remote substituents of both steric hindrance to solvation and the anomeric effect. It is remarkable that such a subtle dependence on substituents, from fine-tuning of the positive charge, can be detected.

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